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PLAGUE EPIZOOTIC AMONG OCHOTONA IN WESTERN MONGOLIA

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Plague epizootics in Western Mongolia near the border of the USSR began to be recorded comparatively not long ago. In 1953, 30 km beyond the frontier with the USSR, in the place called Zak, the culture of Past. pestis was isolated for the first time from the carcass of a marmot (Nekipelov, 1959).

In 1958, in the same area, 20 km from the border of the USSR, a plague epizootic was noted among Alticola (Platycranius) (Demin, 1959; Shamova, 1959).

In 1959, 2 km to the north-east of the place where the plague among Alticola was discovered, in the area between mountains of Khurumtu-Ula and Erzen-Khuren-Ula, an epizootic was ascertained among Ochotona mongolica and Ochotona dahlurica. Here, there were caught and investigated 1008 rodents and 4189 fleas. As a result, 26 cultures of Past. pestis were isolated from the rodents (including 5 cultures from carcasses of O. mongolica) and 25 cultures from the fleas.

The territory described is situated within the limits of the Tsagan-Nur depression belonging to the zone of semideserts (Yunatov, 1950). The basic types of plant formations (Yunatov, 1950; Kalinina, 1948), are finely-cespitose desert steppes with koeleria, wormwood and sheep's fescue, as well as pebbly stipa-wormwood steppes. In the upper strata of the mountains and along their northern slopes alpine meadows are encountered now and then.

O. mongolica is a background species of rodents which inhabit the wormwood-koeleria steppes and cutliers. The Altaian marmot is not numerous, averaging 0.23 of inhabited burrow per hectare. Marmots abound most in the upper part of mountains (up to one inhabited burrow per hectare). Citellus (Urocitellus) undulatus Pall. is not numerous. It is encountered mostly on the riverain terraces. Its density does not exceed 4-5 individuals per hectare. Individual dwellings of O. danurica are distributed over small depressions. Alticola (Platycranius) are usually found among the rocks. Here too, are encountered small numbers of Alticola barakshin Bannikov and Microtus (Stenocranius). Dzhungarian hamsters are also scanty. In the wormwood-stipa steppe dwells Alactaga Sibirica saltator. From among carnivores are common polecat, ermine and fox. Now and then is encountered Felis manul.

The plague-infected rodents were first discovered in the upper part of the range. Here, in June 1959, the cultures of Past. pestis were isolated from the apprehended O. mongolica and from one young marmot. No carcasses of rodents were found. In July, the cultures of Past. pestis were obtained only from O. mongolica, and in August and September, from O. danurica as well. During the same period a mass death was noted among O. mongolica as a result of plague epizootic. During the first two months the epizootic was confined to an area around the place where the plague affected marmot was recovered from a family burrow in which according to our observations had dwelt 6-7 marmots. Subsequently this burrow was found to be deserted, perhaps due to the fact that, as attested by B. M. Kasatkin and V. B. Chekalin (1958), the marmots in case of the death of one of the members of the family abandon their burrow. Thereafter the epizootic began to spread down the valley.

The prevailing species of rodents in the epizootic area was O. mongolica, while other species of rodents were encountered in smaller numbers. The dwellings of O. mongolica in the upper parts of valleys are adapted to plant associations in which wormwood and koeleria predominate. In June the number of inhabited burrows averaged here 20 per hectare. The epizootic among O. mongolica was associated with the period of their high density of population. Towards the autumn the density of O. mongolica decreased in epizootic area to six inhabited burrows per hectare. At the same time, in the safe area their numbers increased 2.5 times.

The infectedness of burrows of pikas with plague in June-July was equal to 20%, whereas the percentage of infected individuals in a burrow reached 30%. In September 40% of burrows were found to be infected with an almost general infectedness

of their inhabitants. The same burrows were infected with plague during the entire warm period although the composition of their inhabitants did not change.

The seasonal infectedness of rodents with plague is shown in Table 1.

TABLE 1

Isolation of Past. pestis Cultures from Rodents by Month

Вид гризуна, от которого выделена культура	1	2	Август	Сентябрь	Итого
Сурок алтайский	—	—	—	—	11
Пищука монгольская	21	32	746	321	17117
Трупы пищух монгольских	—	—	11	44	55
Пищука даурская	—	—	274	18	313
Итого	4	4	10751	833	26136

Note: Denominator -- number of investigated rodents, numerator -- number of isolated cultures.

1 -- Species of the rodent from which the culture was isolated; 2 -- Altaiian marmot; 3 -- O. mongolica; 4 -- Carcasses of O. mongolica; 5 -- O. dahurica; 6 -- June; 7 -- July; 8 -- August; 9 -- September; 10 -- Total.

The burrows of O. mongolica in which their inhabitants died from plague were repopulated either by the Mongolian or Dahurian pikas from the adjacent areas. The repopulation of vacant burrows of O. mongolica by Dahurian pikas could also be observed in other places. In Khalbanur, in July the inhabitants of three burrows of O. mongolica were caught in their entirety. In August two of the burrows were repopulated by Dahurian pikas, and in the third one lived an Urocitellus undulatus. The population of O. Dahurica in the place of the epizootic was not numerous.

In the northern part of epizootic area, in a colony where three plague-stricken carcasses of O. mongolica were gathered, there were also caught two Dahurian pikas and one Dzhungarian hamster, whereupon only one Dahurian pika was found to be free from the causative agent of the plague.

The dying out of the original inhabitants of burrows was accompanied by a migration of fleas onto surviving rodents and onto new settlers. As a result the index of the abundance of fleas on O. mongolica here in the beginning of summer amounted to 3.2 and in the autumn -- to 8.5, with a maximum of 104 fleas. At the same time along with the increase of the number of fleas on O. mongolica the index of the abundance of fleas on O. dahurica also rose sharply (Table 2).

TABLE 2

Comparative Index of Fleas on Ochotona in Various Investigated Areas

Виды грызунов	4. Период обследования	Территория в га	Незараженная территория в га
1. Монгольская пищуха	2. июнь-июль	3.1	3.0
	5. август-сентябрь	1.8	2.1
Даурская пищуха	2. июнь-июль	1.7	1.3
	5. август-сентябрь	13.5	1.4

1 -- Species of rodents; 2 -- O. mongolica; 3 -- O. dahurica; 4 -- Period of investigation; 5 -- Epizootic area in ha; 6 -- Uninfected area in ha; 7 -- June-July; 8 -- August-September.

TABLE 3

Cultures of Past. pestis Isolated From Fleas

Вид грызуна, с которого были сняты чумные блохи	Время исследования				
	V	VI	VII	IX	итого
Пищуха монгольская	4	1	1	5	14
Пищуха даурская	—	—	1	5	6
Суслик длиннохвостый	—	—	—	3	3
Полевка плоскочерепная	—	—	1	1	2
Итого	4	1	6	14	25

1 -- Species of rodents from which plague-infected fleas were recovered; 2 -- O. mongolica; 3 -- O. dahurica; 4 -- Urocyon; 5 -- Alticola platycranus; 6 -- Time of investigation; 7 -- Total.

The fleas were found to be infected with plague during the entire period of the epizootic. Twenty-five cultures of Past. pestis were isolated from pikas, Uroditellus undulatus and Alticola Platycranus (Table 3).

The highest number of cultures was isolated from the fleas in September. The highest number of infected fleas was found on O. mongolica. In the beginning of the epizootic only the fleas of Mongolian pikas were infected, but from August on the infected fleas began to be recovered also from Dahurian pikas. And only in September, when the epizootic had spread over a great area and the number of pikas sharply decreased the infected fleas were also ascertained on susliks and Alticola Platycranus. It is possible that the infected fleas were transferred to Alticola Platycranus from the carcasses of rodents by carnivorous birds, since this Alticola was recovered at two km from the focus of infection, alongside a nest of shrike. The susliks on which the plague-infected fleas were found, were recovered from plague-ridden pika colonies.

To give an idea about the species of fleas from which plague cultures were isolated and of the rodents from which the infected fleas were recovered, as well as of general condition of the rodent at the moment of its arrival in the laboratory, we present the relevant data in Table 4.

Most of the cultures (14) were isolated from fleas Paradoxopsyllus scorodumovi and Amphipsylla runatus J. et R. Almost all cultures from these fleas were obtained in August-September. P. scorodumovi appears in a mass in July, whereas Am. runatus is numerous during the entire warm period. These species of fleas on O. mongolica are prevalent. In all, seven species of fleas were found to be infected: Am. runatus, Frontopsylla hetera W., Ctenophyllus hirticrus J. et R., P. scorodumovi, Amphipsylla primaris J. et R., Rhadinopsylla dahurica J. et R., Rhadinopsylla 11 11 Arg.

In winter, the epizootic was not ascertained. In the spring of 1960, a few cultures of Past. pestis were obtained from still infected fleas recovered from O. mongolica and Dzhungarian hamster. The population of Mongolian pikas in the area swept over by the epizootic at this time sharply decreased and was below that of Dahurian pikas. In the areas of the past epizootic the indexes of the profusion of fleas reached in marmots -- 5.7, on susliks -- 5.3 and on Mongolian pikas -- 32.2. The most infected were fleas removed from O. mongolica; at the entrances of burrows the fleas were encountered in May-June only in the colonies of Mongolian pikas.

TABLE 4

Species of Fleas from which Cultures of *Past. pestis* were Isolated, and Species of Rodents from which these Fleas were Recovered

Вид блох, из которых выделена культура чумного микроба	Вид гризуна, с которого сняты чумные Z-блоки	Состояние гризуна	Количество выделенных культур	Время выделенных культур			
				июнь	июль	август	сентябрь
<i>Amphipsylla runatus</i> J. et R.	1. мышуха монгольская	1. без признаков заболевания	4	—	—	—	4
	2. мышуха монгольская	2. без признаков заболевания	3	2	—	—	1
	3. мышуха даурская	3. без признаков заболевания	1	1	—	—	—
	4. мышуха монгольская	4. без признаков заболевания	10	—	1	4	5
<i>Frontopsylla hetera</i> W. <i>Ctenophyllus lurticus</i> J. et R. <i>Paradoxopsyllus scrodamovi</i> Sc	5. суслик длиннохвостый	5. без признаков заболевания	3	1	—	2	—
	6. мышуха даурская	6. без признаков заболевания	2	—	—	—	—
	7. суслик длиннохвостый	7. без признаков заболевания	2	—	—	—	2
	8. мышуха даурская	8. без признаков заболевания	1	—	—	—	—
<i>Amphipsylla prunaris</i> J. et R. <i>Rhadinopsylla dahurica</i> J. et R.	9. мышуха монгольская	9. без признаков заболевания	1	—	—	—	—
	10. мышуха монгольская	10. без признаков заболевания	1	—	—	—	—
<i>Rhadinopsylla li li</i> Arg	11. мышуха даурская	11. без признаков заболевания	1	—	—	—	—
	12. суслик длиннохвостый	12. без признаков заболевания	1	—	—	—	—

[Key to Table 4]:

1 -- Species of fleas from which cultures of Past. pestis were recovered; 2 -- Species of rodents from which plague-infected fleas were recovered; 3 -- O. mongolica; 4 -- O. dahurica; 5 -- Urocitellus undulatus; 6 -- Alticola platycranus; 7 -- Condition of rodents; 8 -- Sick; 9 -- Without symptoms of plague; 10 -- Carcass; 11 -- Number of isolated cultures; 12 -- Time of isolation of cultures; 13 -- June; 14 -- July; 15 -- August; 16 -- September.

In connection with a great number of cultures isolated from O. mongolica and little susceptibility of guinea pigs to them, it was of interest to ascertain the sensitivity of pikas to the artificial infection with these strains at the moment of their isolation.

The pikas were caught in places where no epizootic was observed and were maintained individually in glass jars. The infection of rodents was effected by a culture isolated from the carcass of a Mongolian pika whose autopsy did not reveal any visible anatomicopathological changes. After 24 hours Petri dishes with agar showed the growth of Past. pestis from all organs, regional lymph node and blood. The colonies were pigmented, granuliform, with a soft colorless lacy zone. In the agar smears -- gram-negative, short rods. A guinea pig, infected subcutaneously with a suspension from internal organs of the pika carcass, survived and was killed by chloroform 10 days after infection. A post mortem showed: right-side regional lymphatic bubo in a period of purulent decomposition, hyperemia of subcutaneous tissue, liver of dark cherry color, all lung lobes evenly hyperemized, spleen affected with necrotic nodules. In the smear-imprints from the lymph node, liver, spleen, lungs and blood from the heart no microflora was detected. In the agar -- abundant growth of typical Past. pestis, from spleen, regional lymph gland and blood. The isolated culture fermented within 24 hours glucose, maltose, rhamnose, galactose, mannitol and xylose, with the formation of acid without gas, whereas it did not ferment lactose, saccharose, dulcitol, arabinose, raffinose, and sorbitol: by the 4th day it fermented glycerol and urea, imparting a slight pinkish color to the medium, did not produce indole, formed hydrogen sulfide and reduced methylene blue within one hour. The reaction of nitrification and denitrification was negative. The culture was lysed by anti-plague and pseudotuberculous phages. Upon titration according to Appelman with a polyvalent plague bacteriophage (series 2, GK 725) the lysis was observed at 10^{-9} (phage titer 10^{-10}), and pseudotuberculous polyvalent bacteriophage (series 1, GK 707) lysed the culture at 10^{-10} (phage titer). The reaction of agglutination was positive with Aktris horse serum (series of 12 March 1960, prepared by the Irkutsk Antiplague Institute),

dilution 1 : 640, titer 1 : 1280. Antigen controls in the experiment did not produce spontaneous agglutination.

The experiment was carried out on 32 pikas -- four each per infecting dose (two males and two females). The animals were selected of the approximately same weight. Guinea pigs and white mice were infected parallelly: 24 rodents of each species, 3 individuals per dose.

All three species of animals were infected at the same time subcutaneously with a suspension of 2-day agar culture.

Results of the experiment are given in Table 5.

TABLE 5

Results of Subcutaneous Infection of O. mongolica

Доза заражения в микробах	Монгольская пика	Морская свинка	Белая мышь
50	4/4	0/3	2/3
500	4/4	0/3	3/3
5 тыс.	4/4	0/3	3/3
50 тыс.	4/4	0/3	2/3
500 тыс.	3/3	0/3	1/3
5 млн.	4/4	0/3	0/3
50 млн.	4/4	1/3	3/3
500 млн.	4/4	0/3	3/3
Всего	21/32	1/24	17/24

Note: Denominator -- number of infected animals, numerator -- number of deaths.

1 -- Infection dose in microbes; 2 -- O. mongolica; 3 -- Guinea pig; 4 -- White mouse; 5 -- thousand; 6 -- million; 7 -- Total.

It may be seen from Table 5 that all pikas, except one, died from plague within 2 to 4 days.

All guinea pigs survived, except one, which died by the 7th day with anatomico-pathological picture characteristic for this disease. From all organs and blood of this guinea pig was isolated Past. pestis. Out of 24 infected white mice, 17 died from plague and 7 survived.

The surviving pika was killed by chloroform and autopsied.

In subcutaneous tissue -- small whitish nodules, regional lymph node enlarged, with a curdled content. Spleen slightly enlarged, crimson colored, cultures from regional lymph node produce the growth of colonies of Past. pestis, having well-pronounced peripheral zone.

Thus, Mongolian pikas proved highly susceptible to experimental infection with Past. pestis isolated from this species of rodents during the epizootic. Doses of 50 microbes and higher prove fatal for them, whereas guinea pigs did not die from 500 million microbes.

The infection in O. mongolica had, as a rule, an acute course of the type of hemorrhagic septicemia. Upon autopsy, the majority of rodents displayed a hyperemia of subcutaneous vessels and tissue with an edema of the latter of different degrees. The regional glands joined with surrounding tissue. In some pikas in the subcutaneous tissue were noted small nodular formations. All internal organs were plethoric, and liver and spleen slightly enlarged. In the smears-imprints from the organs and blood was discovered a great number of bipolarly colored gram-negative rods. Cultures of Past. pestis were isolated from all organs and blood. By their morphological, cultural and biochemical properties they were typical of Past. pestis and did not differ at all from the original strain, except that they reduced faster methylene blue and fermented glycerol more slowly.

The virulence of strains determined according to Reed and Muench is equal to 32 microbes.

The plague infection in guinea pigs ran the course of the type of protracted chronic forms. During the first days after infection the guinea pigs were seriously ill but after six days their condition improved abruptly. Two weeks after their inoculation with plague all the 23 guinea pigs were killed by chloroform. Upon autopsy, in 20 guinea pigs were found inguinal buboes in the process of purulent or curdled decomposition. The spleen was slightly enlarged with necrotic nodules. In the smears-imprints from the organs no microflora was found. From all chloroformed guinea pigs Past. pestis was isolated only from the regional lymph node.

White mice died from plague within three to eight days with an anatomicopathological picture characteristic of this disease. Past. pestis was isolated from all organs. From five mice out of seven which survived Past. pestis was also obtained (from regional lymph node).

Thus, the experimental data confirmed the high sensitivity of Mongolian pikas to plague strains discovered in them during the epizootic, whereas for the guinea pigs these strains proved to be weakly virulent. In the spring the population of Mongolian pikas became very low, whereas that of Dahurian pikas was higher. The epizootic ended apparently in consequence of dying out of Mongolian pikas and repopulation of infected burrows by less susceptible under given conditions species of rodents, such as *Urocitellus undulatus* and *O. dahurica*. We may also assume that Mongolian pikas received infection from a sick marmot, inasmuch as the first infected individuals were found near its burrow and it was here within a limited area that the epizootic ran its course during the first two months.

The nearness of the epizootic focus to the frontiers of the USSR and the absence of natural barriers for the immigration of rodents from the territory of the Mongolian People's Republic dictate the necessity of strengthening antiepidemic measures in the areas of the Gorno-Altai autonomous region adjacent to Western Mongolia.

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